Effects of dietary selenium supply and timing of nutrient restriction during gestation on maternal growth and body composition of pregnant adolescent ewes[1]

D. B. Carlson,* J. J. Reed,* P. P. Borowicz,* J. B. Taylor,† L. P. Reynolds,* T. L. Neville,* D. A. Redmer,* K. A. Vonnahme,* and J. S. Caton*[2]

*Center for Nutrition and Pregnancy, Department of Animal and Range Science, North Dakota State University, Fargo 58105; and †USDA-ARS, US Sheep Experiment Station, Dubois, ID 83423

ABSTRACT: The objectives were to examine effects of dietary Se supplementation and nutrient restriction during defined periods of gestation on maternal adaptations to pregnancy in primigravid sheep. Sixty-four pregnant Western Whiteface ewe lambs were assigned to treatments in a 2 × 4 factorial design. Treatments were dietary Se [adequate Se (ASe; 3.05 μg/kg of BW) vs. high Se (HSe; 70.4 μg/kg of BW)] fed as Se-enriched yeast, and plane of nutrition [control (C; 100% of NRC requirements) vs. restricted (R; 60% of NRC requirements]. Selenium treatments were fed throughout gestation. Plane of nutrition treatments were applied during mid (d 50 to 90) and late gestation (d 90 to 130), which resulted in 4 distinct plane of nutrition treatments [treatment: CC (control from d 50 to 130), RC (restricted from d 50 to 90, and control d 90 to 130), CR (control from d 50 to 90, and restricted from d 90 to 130), and RR (restricted from d 50 to 130)]. All of the pregnant ewes were necropsied on d 132 ± 0.9 of gestation (length of gestation ≈145 d). Nutrient restriction treatments decreased ewe ADG and G:F, as a result, RC and CR ewes had similar BW and maternal BW (MBW) at necropsy, whereas RR ewes were lighter than RC and CR ewes. From d 90 to 130, the HSe-CC ewes had greater ADG (Se × nutrition; $P = 0.05$) than did ASe-CC ewes, whereas ADG and G:F (Se × nutrition; $P = 0.08$) were less for HSe-RR ewes compared with ASe-RR ewes. The CR and RR treatments decreased total gravid uterus weight ($P = 0.01$) as well as fetal weight ($P = 0.02$) compared with RC and CC. High Se decreased total (g; $P = 0.09$) and relative heart mass (g/kg of MBW; $P = 0.10$), but increased total and relative mass of liver ($P \leq 0.05$) and perirenal fat ($P \leq 0.06$) compared with ASe. Total stomach complex mass was decreased ($P < 0.01$) by all the nutrient restriction treatments, but was reduced to a greater extent in CR and RR compared with RC. Total small intestine mass was similar between RC and CC ewes, but was markedly reduced ($P < 0.01$) in CR and RR ewes. The mass of the stomach complex and the small and large intestine relative to MBW was greater ($P = 0.01$) for RC than for CR ewes. Increased Se decreased jejunal DNA concentration ($P = 0.07$), total jejunal cell number ($P = 0.03$), and total proliferating jejunal cell number ($P = 0.05$) compared with ASe. These data indicate that increased dietary Se affected whole-body and organ growth of pregnant ewes, but the results differed depending on the plane of nutrition. In addition, the timing and duration of nutrient restriction relative to stage of pregnancy affected visceral organ mass in a markedly different fashion.

Key words: fetal, maternal, nutrient restriction, pregnancy, selenium

J. Anim. Sci. 2009. 87:669–680
doi:10.2527/jas.2007-0837

INTRODUCTION

Selenium is essential for normal growth and development in livestock (Underwood and Suttle, 2001); therefore, Se is typically supplemented in livestock diets at concentrations that do not exceed 0.3 mg/kg of diet (FDA, 2004). Research in sheep and cattle has demonstrated that dietary Se provided as Se-enriched yeast can be fed at concentrations 20 times greater than 0.3 mg/kg of diet with no signs of toxicity (Juniper et al., 2008).

[1]This project was partially supported by National Research Initiative Competitive Grants No. 2003-35206-13621 and 2005-35206-15281 from the USDA Cooperative State Research, Education, and Extension Service, and partially by NIH Grant HL 64141. Gratitude is expressed to employees of the Animal Nutrition and Physiology Center and Ruminant Nutrition and Physiology Laboratories for their contributions to this project.

[2]Corresponding author: joel.caton@ndsu.edu

Received December 29, 2007.
Accepted October 17, 2008.

Supranutritional levels of dietary Se have been shown to inhibit cell proliferation, angiogenesis, and tumor growth in rodent cancer models (Lu and Jiang, 2001; Zeng and Combs, 2008). Based on the numerous possible impacts of dietary Se, it is important to investigate the effects of supranutritional levels of Se on growth and development of healthy tissues in meat animals, particularly during disparate physiological states such as pregnancy and nutrient deprivation.

In sheep, advancing pregnancy is accompanied by increases in visceral tissue mass (Scheaffer et al., 2004a), as well as increases in jejunal vascularity and total microvascular volume (Scheaffer et al., 2004b). Previous reports have examined the effects of nutrient restriction (Scheaffer et al., 2004a,b) or the interaction of Se supplementation and nutrient restriction (Reed et al., 2007) during the last two-thirds of ovine pregnancy. Recently, Reed et al. (2007) reported that Se supplementation of pregnant, primigravid ewes increased fetal weight; however, Se supplementation did not alter maternal organ mass, jejunal cellularity, or jejunal vascularity. However, the effects of dietary Se supplementation and nutrient restriction during mid and late gestation on maternal adaptations to pregnancy are unclear. The objective of this study was to determine the effects of Se supplementation and nutrient restriction during defined periods of gestation (mid, late, or both) and on growth and development of maternal organs in pregnant, primigravid sheep.

MATERIALS AND METHODS

All experimental protocols were approved by the North Dakota State University Animal Care and Use Committee.

Animal Management and Treatments

Western Whiteface ewe lambs originated from the US Sheep Experiment Station (Dubois, ID) flock. Initially, estrus-synchronized ewe lambs were exposed to rams for 72 h. Following breeding, rams were removed and ewes were randomly assigned to 2 separate pens, and pens were assigned randomly to an adequate (**ASe**) or high (**HSe**) dietary Se treatment. Ewes were pen-fed a basal diet (2.04 kg/ewe daily) that contained (DM basis) 47% alfalfa hay, 20% corn, 20% sugarbeet pulp pellets, 8% malt barley straw, and 5% concentrated separator byproduct (desugared molasses). In addition to the basal diet, ewes assigned to the ASe treatment were fed 100 g/d of a control pellet that was balanced to contain 0.30 mg/kg of Se, whereas HSe ewes were fed 100 g/d of a high-Se pellet balanced to contain 47.5 mg/kg of Se, provided as Se-enriched yeast (Sel-Plex, Alltech, Nicholasville, KY). The control and high-Se pellets were formulated using similar ingredients to maintain similar concentrations of ME, CP, ADF, NDF, Ca, and P. Selenium-enriched yeast replaced soybean meal and partially replaced ground corn relative to the control pellet. The approach by which dietary Se was supplemented to pregnant, primigravid ewes has been used previously by our laboratory (Reed et al., 2007). The ingredient and nutrient composition of the control and high-Se pellets is presented in Table 1.

Table 1. Ingredient and chemical composition (DM basis) of the control and high-Se pellet fed to ewes

Item, % of dietary DM	Control pellet	Se pellet
Ingredient		
Beet pulp	36.5	36.5
Alfalfa meal	22.3	22.3
Corn	18.2	16.2
Soybean hulls	18.0	18.0
Soybean meal, 48%	5.0	—
Se-enriched yeast[1]	—	7.0
Chemical composition		
CP, %	13.7	13.5
ADF, %	24.3	24.7
NDF, %	39.8	41.1
Ca, %	0.68	0.68
P, %	0.22	0.25
Cu, mg/kg	12.54	11.60
Se, mg/kg	0.23	40.95
ME,[2] Mcal/kg	2.66	2.71

[1]Sel-Plex (Alltech, Nicholasville, KY).
[2]Estimated using values obtained from the NRC (1985).

Fetal counts were estimated by ultrasonography using a rectal probe (Aloka, Wallingford, CT) in each ewe on d 32 after breeding. Sixty-four ewes (50.7 ± 2.8 kg of BW) estimated to have a single fetus were selected to remain on the ASe or HSe treatment and were subsequently transported (40 d after breeding) to the Animal Nutrition and Physiology Center at North Dakota State University for the remainder of the experiment.

At North Dakota State University, ewes were housed in individual pens (0.91 × 1.2 m) in an indoor facility until necropsy at d 132 ± 0.9 of gestation. Within the facility, the temperature was held constant at 12°C, and lighting was controlled automatically to mimic the photoperiod of the outdoor environment. All ewes had access to fresh water and trace mineralized salt that contained no added Se (American Stockman, Overland Park, KS).

Stage of gestation for each ewe was estimated using average day of breeding. On d 50 of gestation, ewes within each Se treatment were stratified by average breeding date and assigned to 1 of 4 distinct plane of nutrition treatments. Ewes were offered diets that were balanced to meet 100% [control (**C**)] or 60% [restricted (**R**)] of predicted ME requirements of pregnant ewe lambs (NRC, 1985). The plane of nutrition treatments were applied from d 50 to 90 (mid gestation) and d 90 to 130 (late gestation), which resulted in 4 distinct treatment combinations designated by **CC** (control from d 50 to 130), **RC** (restricted from d 50 to 90, and control d 90 to 130), **CR** (control from d 50 to 90, and restricted from d 90 to 130), and **RR** (restricted from d 50 to 130).

During mid and late gestation, ewes assigned to the ASe treatment derived all dietary nutrients from the control pellet. The HSe ewes were fed the high-Se pellet at a rate that met the desired Se intake (70.4 μg/kg of BW), and the remainder of the diet was composed of the control pellet to achieve desired ME intake. Ewes were weighed every 14 d, and intakes of the control and high-Se pellet were adjusted based on ewe BW and stage of gestation. This approach allowed dietary Se intake to be held constant relative to BW for HSe ewes, but Se intake varied with DMI in ASe ewes. Nutrient requirements were based on recommendations for 60-kg ewes in mid or late gestation (weighted ADG of 140 g; NRC, 1985). Dry matter intake was determined daily by weighing and recording the amount of feed offered and refused. Refusals rarely occurred. Individual ingredients and pelleted diets were sampled each time a new batch of pellets was received. Feed samples were analyzed for DM, ash, CP, Ca, and P (methods 930.15, 942.05, 990.02, 968.08, and 965.17, respectively, AOAC, 1990), NDF, ADF (Ankom Technology, Macedon, NY), and Se (Finley et al., 1996).

Maternal Necropsy Procedures

Fetal age was estimated according to average breeding date, which was then used to assign a necropsy date for each ewe resulting in an average gestation length of 132 ± 0.9 d. On the morning of necropsy, ewes were weighed to determine their final BW. A jugular blood sample (10 mL) was collected into sterile evacuated tubes containing EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Plasma was obtained by centrifugation (1,500 × *g* for 30 min) and stored at −20°C until further analysis of plasma Se by atomic absorption spectrometry (Finley et al., 1996). Exactly 1 h before necropsy, ewes were injected with 5-bromo-2-deoxy-uridine (**BrdU**; 5 mg/kg of BW) via jugular venipuncture to evaluate the rate of jejunal cell proliferation (described below). Each ewe was stunned by captive bolt (Supercash Mark 2, Accles and Shelvoke Ltd., Birmingham, UK) and exsanguinated; blood was subsequently captured and weighed. The gravid uterus (including cervix) was immediately dissected and weighed. Fetuses were removed from the placenta, and fetal BW was measured. The ewe was eviscerated and the viscera (including digesta) weighed.

Dissection and sampling of maternal organs and tissues were conducted as described previously (Scheaffer et al., 2004a; Reed et al., 2007). After removal of the viscera, the heart, lungs, kidneys, adrenals, and perirenal fat were removed from the body cavity. In addition, the liver, spleen, and pancreas were dissected from the viscera. The stomach complex was separated from the esophagus at the cardia and from the intestine at the pylorus, and subsequently separated into the rumen, reticulum, omasum, and abomasum. Omental and mesenteric fat was separated from all visceral tissues.

The small intestine was segmented into duodenum, jejunum, and ileum, as described previously (Soto-Navarro et al., 2004; Reed et al., 2007). Briefly, the duodenum was identified as the segment that extended from the pylorus to a point directly adjacent to the entry of the gastrosplenic vein into the mesenteric vein. Beginning at the mesenteric and ileocecal vein junction, a 15-cm measurement was made caudally along the mesenteric vein, and the mesenteric vasculature was followed to the point of intestinal intersection. From this point, a 150-cm measurement was made caudally along the small intestine, and this section of jejunum was removed. Approximately 30-cm of the 150-cm jejunal section was removed for further analysis, as described below, with the remainder of the 150-cm section used for vascular perfusion (described below). An additional 150-cm section was measured caudal to the excised section and served as the terminal end of the jejunum. The remainder of the jejunum comprised the section that was cranial to the section removed for perfusion. The ileum was defined as the segment between the terminal end of the jejunum and the ileocecal junction. After identification of the small intestinal segments, the intestine was separated from the mesentery, the digesta was carefully removed, and the segments were weighed. The large intestine was removed and processed in a similar fashion. Individual organ and tissue weights were determined after dissection and removal of digesta. Carcass weight, including head, hide, and hooves, was determined after removal of internal organs and tissues.

Cellularity Estimates

Samples of jejunum and jejunal mucosa were obtained as described previously (Reed et al., 2007). For jejunal mucosal sampling, a subsample (5 cm) of the 30-cm jejunal tissue sample was gently washed in PBS buffer, weighed, placed on a polyethylene cutting board, and opened with the lumen side up. Mucosal tissue was separated (scraped) from the remaining tissues with a glass histological slide, and the remaining jejunal tissue was weighed. A portion of jejunum and jejunal mucosa was stored at −80°C and analyzed for concentrations of DNA (Johnson et al., 1997), RNA (Reynolds et al., 1990), and protein (Bradford, 1976), as described elsewhere (Reed et al., 2007). Concentration of DNA was used as an index of hyperplasia (cell number), whereas protein:DNA and RNA:DNA ratios were used as indices of hypertrophy (cell size) and potential metabolic capacity per cell, respectively.

Jejunal Cell Proliferation

Subsamples of jejunum and jejunal mucosa were immersed in Carnoy's fixative (60% ethanol, 30% chloroform, 10% glacial acetic acid) for 3 h and then transferred to a 70% ethanol solution. Fixed tissues were embedded in paraffin, sectioned (4 μm), and mounted

on glass slides using standard histological techniques (Luna, 1968). Proliferating cells (S-phase of the cell cycle) were identified immunohistochemically, as reported previously (Jablonka et al., 1991; Swanson et al., 1999; Reed et al., 2007). Briefly, the tissue section was rehydrated and then was incubated with mouse anti-BrdU (Clone BMC 9318, Roche Diagnostics, Indianapolis, IN). Positive staining of the primary antibody was detected using 3, 3′-diaminobenzidine (Vector Laboratories, Burlingame, CA). Hematoxylin (EMD Chemicals Inc., Gibbstown, NJ) was used to counterstain the nondividing nuclei, and periodic acid-Schiff's staining procedure (Luna, 1968) was utilized to highlight other structures present within the jejunal tissue cross-section. The relative rate of cellular proliferation (labeling index, which represents the proportion or percentage of cells proliferating) was quantified using Image-Pro Plus 5.0 software (MediaCybernetics Inc., Silver Spring, MD).

Small Intestine Vascularity

A portion of the freshly excised jejunum was perfusion fixed with Carnoy's as described by Soto-Navarro et al. (2004), with the exception that a different casting resin was used, as described by Reed et al. (2007). Briefly, a latex resin [Microfil MV-132 (4 mL of latex compound combined with 5 mL of diluents), Flow Tech Inc., Carver, MA] was used as the casting resin. Cross-sections of perfused jejunal tissue were processed as described above. Tissue sections (4 μm) were stained using periodic acid-Schiff's staining procedures (Luna, 1968) to provide contrast to the vascular tissue. Mean capillary area, capillary number, and capillary circumference were measured in the intestinal villi using Image-Pro Plus 5.0 software (MediaCybernetics), as described previously (Soto-Navarro et al., 2004; Reed et al., 2007).

Calculations and Statistical Analysis

Maternal BW (**MBW**) was calculated as BW minus the sum of the weights of the digesta and the gravid uterus. Maternal organs and tissues are expressed either as fresh tissue weight (g or kg) or weight relative to MBW (g/kg). Carcass weight was calculated as the weight of head, hide, and carcass after removal of all internal organs. Percentage jejunal mucosa was calculated by dividing the mucosal scrape mass by the sample mass before scraping. Total jejunal mucosa was calculated by multiplying the percentage mucosa by total jejunal mass. Total tissue content of DNA, RNA, and protein was calculated by multiplying the analyzed concentration by wet tissue weight. Ratios of RNA:DNA and protein:DNA were determined from values for DNA, RNA, and protein concentrations (mg/g).

Percentage proliferating cells was estimated by dividing the number of 3, 3′-diaminobenzidine-stained (BrdU-positive) nuclei by the total number of nuclei present within the area of tissue analyzed. Number of proliferating cells was calculated by dividing total tissue DNA (g) by 6.6×10^{-12} g (Baserga, 1985) and then multiplying that value by percentage proliferating cells.

Capillary area density was determined by dividing the total capillary area by the area of tissue analyzed. Capillary number density was calculated by dividing the total number of vessels counted by the tissue area evaluated. To estimate the capillary surface density (total capillary circumference per unit of tissue area), mean capillary perimeter (circumference) was divided by tissue area evaluated (Borowicz et al., 2007; Reed et al., 2007). Although capillary surface density actually represents the average of the circumference of the capillary cross-sections, it is nevertheless proportional to their surface area (Borowicz et al., 2007). Finally, mean area per capillary was determined by dividing total capillary area by the number of capillaries within the tissue area evaluated. Total vascularity (mL) of jejunum and jejunal mucosa was calculated by multiplying the capillary area density (%) by tissue mass (g), as described previously (Soto-Navarro et al., 2004; Reed et al., 2007).

The data were arranged as a 2 × 4 factorial and were analyzed as a completely randomized design using the GLM procedure (SAS Inst. Inc., Cary, NC). Factors were amount of dietary Se and plane of nutrition during mid and late gestation. The interaction of Se and plane of nutrition were also included in the model. The number of fetuses carried by each ewe was included in the model for all variables and was retained in the model if significant ($P \leq 0.10$). Main effects of treatments and the interaction were deemed significant at $P \leq 0.10$ (in an effort to emphasize biology), and specific observed significance levels are listed in the tables. Means associated with a significant F-test were separated by least significant difference, and significance was declared at $P \leq 0.10$. Means associated with a significant interaction are presented in the text.

RESULTS

Ewe BW, DMI, and Growth Performance

From d 50 to 130, Se intake (μg/kg of BW) differed due to Se supplementation ($P < 0.01$) as well as plane of nutrition ($P < 0.01$). Selenium intake was 3.52, 3.20, 3.05, and 2.42 μg/kg of BW for ASe-CC, ASe-RC, ASe-CR, and ASe-RR ewes, respectively, compared with 70.9, 70.6, 70.4, and 69.9 μg/kg of BW for HSe-CC, HSe-RC, HSe-CR, and HSe-RR ewes, respectively. As a result of Se supplementation, HSe ewes had greater ($P < 0.01$) plasma Se concentration on d 130 compared with the ASe treatment (0.62 vs. 0.21 ± 0.03 μg/mL). Plasma Se concentration did not differ due to plane of nutrition ($P = 0.63$) and Se × nutrition ($P = 0.54$) treatments on d 130.

Least squares means for ewe growth performance are presented in Table 2. Dietary Se alone did not affect

Table 2. Least squares means for BW, ADG, DMI, and G:F as influenced by dietary Se and plane of nutrition during mid and late gestation in pregnant adolescent ewes

	Selenium[1]			Nutrition[2]					*P*-value[3]		
Item	ASe	HSe	SEM	CC	RC	CR	RR	SEM	Se	Nutrition	Se × nutrition
BW, kg											
d 50	50.1	49.8	0.6	52.4[a]	47.6[c]	50.4[b]	49.5[b]	0.9	0.67	0.01	0.77
d 90	43.5	53.6	0.6	58.4[a]	48.7[b]	56.6[a]	50.6[b]	1.0	0.89	<0.01	0.95
d 130	59.9	59.7	0.7	67.2[a]	58.8[b]	59.5[b]	53.5[c]	1.1	0.82	<0.01	0.18
ADG, g/d											
d 50 to 90	76.0	85.3	7.9	138.6[a]	23.8[b]	137.6[a]	22.8[b]	12.1	0.37	<0.01	0.66
d 90 to 130	158.6	150.9	12.9	221.1[a]	253.4[a]	72.6[b]	71.8[b]	19.7	0.65	<0.01	0.05
d 50 to 130	115.6	115.9	5.4	178.1[a]	129.1[b]	108.8[c]	47.0[d]	8.2	0.98	<0.01	0.13
DMI, g/d											
d 50 to 90	711.4	709.0	4.6	906.6[a]	515.4[c]	892.7[b]	526.2[c]	7.0	0.68	<0.01	0.88
d 90 to 130	716.8	705.5	9.7	878.6[a]	890.2[a]	542.9[b]	533.0[b]	14.8	0.37	<0.01	0.63
d 50 to 130	708.8	701.8	6.2	890.9[a]	704.7[b]	695.9[b]	529.6[c]	9.5	0.39	<0.01	0.51
G:F											
d 50 to 90	0.09	0.11	0.01	0.15[a]	0.05[b]	0.16[a]	0.04[b]	0.02	0.21	<0.01	0.52
d 90 to 130	0.21	0.19	0.02	0.25[a]	0.28[a]	0.13[b]	0.13[b]	0.03	0.64	0.01	0.08
d 50 to 130	0.16	0.16	0.01	0.20[a]	0.18[a]	0.16[b]	0.09[c]	0.01	0.99	<0.01	0.14

[a–d]Means within a row without a common superscript differ ($P \leq 0.10$).

[1]Adequate Se (ASe, 3.05 μg/kg of BW) and high Se (HSe, 70.4 μg/kg of BW) treatments were applied from breeding until slaughter (~d 132 of gestation).

[2]Nutritional treatments were control (C; 100% of maintenance energy requirements) or restricted (R; 60% of maintenance energy requirements). Treatment combinations were: CC (control from d 50 to 130), RC (restricted from d 50 to 90, and control d 90 to 130), CR (control from d 50 to 90, restricted from d 90 to 130), and RR (restricted from d 50 to 130).

[3]Probability values for effects of Se, nutrition, and their interaction.

BW, ADG, DMI, or G:F at any point during d 50 to 130 of gestation; however, Se × nutrition treatment interactions existed for ADG and G:F from d 90 to 130, which are discussed below. On d 50, BW differed slightly among nutritional treatments ($P = 0.01$) such that CC ewes were heavier than all other treatments, and CR and RR ewes were heavier than RC ewes. However, ewe BW on d 50 did not differ between dietary Se treatments ($P = 0.67$) or among Se × plane of nutrition treatments ($P = 0.77$).

As intended by study design, RC and RR ewes had less ($P < 0.01$) average DMI from d 50 to 90 than did the CC and CR treatments, resulting in decreased ADG ($P < 0.01$) and G:F ($P < 0.01$). From d 50 to 90, CR ewes had slightly less DMI than CC ewes because CR ewes were slightly lighter than CC ewes on d 50, and the amount of DM offered was determined based on BW. On d 90, nutrient-restricted ewes (RC and RR) were lighter ($P < 0.01$) than ewes fed to requirements (CC and CR), and no differences in BW existed between CC vs. CR as well as RC vs. RR.

As expected, CR and RR ewes had less DMI ($P < 0.01$), ADG ($P < 0.01$), and G:F ($P < 0.01$) compared with CC and RC ewes from d 90 to 130. Significant Se × nutrition interactions existed for ADG ($P = 0.05$) and G:F ($P = 0.08$) from d 90 to 130; HSe-CC ewes had greater ADG than did ASe-CC ewes (247 vs. 195 ± 22 g/d), whereas HSe-RR ewes had less ADG (44 vs. 99 ± 22 g/d) and G:F (0.08 vs. 0.18 ± 0.04) compared with ASe-RR ewes. On d 130, CC ewes were heavier ($P < 0.01$) than all other nutritional treatments, RC and CR ewes were of similar BW, and RR ewes were lighter than all other nutritional treatments. From d 50 to 130, ADG differed ($P < 0.01$) among all plane of nutrition treatments. As expected, ADG was greatest for CC ewes and least for RR ewes, whereas RC had greater ADG than did CR ewes. Average G:F from d 50 to 130 was similar for CC and RC ewes, but CR ewes exhibited poorer efficiency of BW gain than did RC ewes.

Maternal Body Composition

Least squares means for carcass weight, MBW, gravid uterus weight, digesta weight, and fetal weight are presented in Table 3. Selenium supplementation did not alter ($P \geq 0.25$) any of these measurements. Carcass weight ($P < 0.01$) and MBW ($P < 0.01$) were less for nutrient-restricted ewes compared with CC ewes, whereas RC and CR ewes had heavier carcass weight and MBW than RR ewes. The CC and RC ewes had greater ($P = 0.01$) total gravid uterus mass than CR and RR ewes; however, gravid uterus mass relative to MBW was not affected ($P = 0.14$) by nutrient restriction. Fetal weight was less ($P = 0.02$) for CR and RR compared with CC ewes, whereas fetal weight for RC ewes was similar to all other plane of nutrition treatments.

Least squares means for maternal blood and organ masses determined at necropsy are presented in Table 4. Blood mass (g and g/kg of MBW) was not affected by Se supplementation ($P \geq 0.31$) or nutrient restriction ($P \geq 0.39$). At necropsy, relative lung mass was greater ($P = 0.01$) in RR ewes compared with all other

Table 3. Least squares means for weight of carcass, maternal body, digesta, gravid uterus, and fetuses as influenced by dietary Se and plane of nutrition during mid and late gestation in pregnant adolescent ewes

	Selenium[1]			Nutrition[2]					*P*-value[3]		
Item	ASe	HSe	SEM	CC	RC	CR	RR	SEM	Se	Nutrition	Se × nutrition
Carcass,[4] kg	36.0	35.6	0.6	40.7[a]	34.8[b]	36.1[b]	31.6[c]	1.0	0.66	<0.01	0.91
MBW,[5] kg	46.5	46.3	0.7	52.3[a]	45.1[b]	47.1[b]	41.2[c]	1.1	0.87	<0.01	0.75
Digesta,[6] kg	6.14	5.70	0.32	6.44	6.26	5.46	5.52	0.49	0.31	0.20	0.10
g/kg of MBW	134.3	123.5	7.2	123.8	141.3	116.0	134.6	10.9	0.25	0.27	0.13
Gravid uterus, kg	9.16	9.10	0.33	9.72[a]	9.50[a]	8.72[b]	8.58[b]	0.43	0.84	0.01	0.43
g/kg of MBW	198.1	198.7	8.8	189.9	210.0	188.0	205.6	11.4	0.95	0.14	0.78
Fetal weight, kg	3.62	3.52	0.14	3.92[a]	3.57[ab]	3.41[b]	3.38[b]	0.20	0.48	0.02	0.49

[a–c]Means within a row without a common superscript differ ($P \leq 0.10$).

[1]Adequate Se (ASe, 3.05 μg/kg of BW) and high Se (HSe, 70.4 μg/kg of BW) treatments were applied from breeding until slaughter (~d 132 of gestation).

[2]Nutritional treatments were control (C; 100% of maintenance energy requirements) or restricted (R; 60% of maintenance energy requirements). Treatment combinations were: CC (control from d 50 to 130), RC (restricted from d 50 to 90, and control d 90 to 130), CR (control from d 50 to 90, restricted from d 90 to 130), and RR (restricted from d 50 to 130).

[3]Probability values for effects of Se, nutrition, and their interaction.

[4]Carcass (head, hide, and carcass) = final BW − total internal organs.

[5]MBW = maternal BW = final BW − (digesta + gravid uterus).

[6]g/kg of MBW = organ mass (g)/MBW (kg).

plane of nutrition treatments. Heart mass (g) was reduced ($P = 0.01$) in nutrient-restricted ewes relative to CC ewes. In addition, the HSe treatment decreased total heart mass ($P = 0.09$) and heart mass relative to MBW ($P = 0.10$) compared with ASe.

Total visceral tissue mass (including digesta) was less ($P < 0.01$) in RC versus CC ewes, but total visceral tissue mass was further decreased by CR and RR treatments compared with RC and CC ewes. Spleen mass was less ($P = 0.02$) in RR ewes compared with CC and CR ewes, but was similar among RC ewes and other treatments. Pancreas mass (g) was decreased ($P = 0.01$) by CR and RR relative to CC and RC treatments. Additionally, relative pancreas mass (g/kg of MBW) was least ($P = 0.02$) for CR compared with RC and RR ewes. Ewes fed the HSe diet had greater total ($P = 0.02$) and relative ($P = 0.05$) liver mass compared with ewes fed the ASe diet. Plane of nutrition treatments markedly affected ($P < 0.01$) total liver mass, such that CC > RC > CR > RR, whereas relative liver mass was greater ($P = 0.03$) for RC compared with other treatments.

Total stomach complex mass was decreased ($P < 0.05$) by all nutrient restriction treatments; however, CR and RR further decreased total stomach complex mass compared with the RC treatment. Relative stomach complex mass was less ($P < 0.01$) for CR ewes compared with RC and RR ewes. Omental and mesenteric fat mass was decreased ($P = 0.01$) to a similar degree in nutrient-restricted ewes compared with CC ewes, although omental and mesenteric fat mass relative to MBW was similar ($P = 0.93$) among all treatments. Similarly, nutrient restriction decreased ($P = 0.06$) total perirenal fat mass, but relative perirenal fat mass was unaffected ($P = 0.85$). Interestingly, the HSe treatment increased total ($P = 0.06$) and relative ($P = 0.04$) perirenal fat mass compared with ASe ewes. Total kidney mass was less ($P = 0.01$) for nutrient-restricted ewes compared with CC ewes, although total kidney mass was decreased further in RR versus RC ewes. Relative kidney mass was similar ($P = 0.13$) among plane of nutrition treatments. Total ($P \geq 0.72$) and relative ($P \geq 0.32$) adrenal gland mass were not altered by Se supplementation or nutrient restriction. Total mammary gland mass was decreased ($P = 0.06$) by CR and RR treatments compared with RC and CC, whereas relative mammary gland mass was not altered ($P = 0.19$) by nutrient restriction.

Data for intestinal mass are presented in Table 5. Effects of Se and Se × nutrition interactions were not significant ($P \geq 0.12$) for any intestinal mass measurement. Total mass of the small intestine ($P < 0.01$), jejunum ($P = 0.09$), and ileum ($P = 0.01$) followed a similar trend among plane of nutrition treatments, such that the mass of these organs in CC and RC ewes was greater than CR and RR ewes. When expressed relative to MBW, the RC and RR ewes had greater small intestinal ($P = 0.01$) and jejunal ($P = 0.05$) mass compared with CC and CR ewes. Neither dietary Se nor plane of nutrition treatments affected the proportion of jejunal mucosal relative to total jejunal mass ($P \geq 0.64$) or total jejunal mucosal mass ($P \geq 0.11$), although CR ewes had less ($P = 0.08$) jejunal mucosa relative to MBW than did RC and RR ewes. Relative ileal mass was greater ($P = 0.01$) for RC ewes compared with other treatments, whereas relative ileal mass was less in CR compared with CC ewes. Dietary Se, nutrient restriction, and combinations did not alter total ($P \geq 0.22$) or relative ($P \geq 0.31$) duodenal mass. Total large intestinal mass was decreased ($P = 0.01$) by all nutrient restriction treatments, but CR ewes had less large intestinal mass than did RC ewes. Large intestinal

mass relative to MBW was decreased ($P = 0.01$) by the CR treatment compared with other plane of nutrition treatments.

Maternal Jejunal Cellularity

Least squares means for concentration and total content of DNA, RNA, protein, and associated ratios (RNA:DNA and protein:DNA) in jejunum and jejunal mucosa are detailed in Table 6. Ewes fed the HSe diet had reduced ($P = 0.07$) jejunal DNA concentration compared with ASe ewes, which resulted in less ($P = 0.03$) total jejunal DNA content. Jejunal DNA concentration was greater ($P = 0.07$) for CR ewes compared with all other plane of nutrition treatments, although total jejunal DNA content did not differ ($P = 0.12$) due to changes in total jejunal mass. Jejunal mucosal DNA concentration ($P = 0.06$), DNA content ($P = 0.08$), and RNA concentration ($P = 0.04$) were greater for CR than for RR ewes, but were equivalent between CC and RC ewes. In addition, the RR treatment decreased jejunal mucosal DNA concentration and total DNA content compared with the CC treatment. Additional measurements of cellularity, cell size, and cellular activity in the

Table 4. Least squares means for maternal blood and organ weights as influenced by dietary Se and plane of nutrition during mid and late gestation in pregnant adolescent ewes

	Se[1]			Nutrition[2]					*P*-value[3]		
Item	ASe	HSe	SEM	CC	RC	CR	RR	SEM	Se	Nutrition	Se × nutrition
Blood,[4,5] kg	2.23	2.41	0.16	2.57	2.30	2.31	2.11	0.25	0.40	0.39	0.89
g/kg of MBW	48.0	52.5	3.4	49.0	51.1	49.6	51.4	5.2	0.31	0.97	0.74
Lung, g	504.5	517.9	26.0	516.0	459.8	520.7	548.2	39.7	0.69	0.38	0.50
g/kg of MBW	10.8	11.3	0.6	9.9^{b}	10.1^{b}	11.1^{b}	13.3^{a}	0.8	0.49	0.01	0.49
Heart, g	232.7	219.3	6.1	251.1^{a}	221.6^{bc}	226.0^{b}	205.2^{c}	9.3	0.09	0.01	0.35
g/kg of MBW	5.03	4.74	0.13	4.81	4.93	4.81	4.99	0.20	0.10	0.79	0.53
Full viscera,[6] kg	10.7	10.5	0.39	12.1^{a}	11.2^{b}	9.69^{c}	9.53^{c}	0.50	0.53	<0.01	0.37
g/kg of MBW	243.3	235.4	6.9	237.0^{b}	261.7^{a}	215.6^{c}	243.2^{ab}	10.5	0.38	0.01	0.54
Spleen, g	90.0	88.2	4.3	99.6^{a}	86.7^{ab}	92.9^{a}	77.2^{b}	6.6	0.75	0.02	0.68
g/kg of MBW	1.93	1.92	0.10	1.91	1.93	1.99	1.88	0.15	0.91	0.94	0.67
Pancreas, g	63.9	59.7	2.5	67.9^{a}	68.7^{a}	54.9^{b}	55.6^{b}	3.8	0.20	0.01	0.39
g/kg of MBW	1.38	1.30	0.06	1.30^{bc}	1.52^{a}	1.17^{c}	1.37^{ab}	0.08	0.28	0.02	0.17
Liver, g	544.9	581.6	11.65	640.2^{a}	587.2^{b}	547.1^{c}	478.7^{d}	17.8	0.02	<0.01	0.75
g/kg of MBW	12.3	13.1	0.4	12.7^{b}	13.7^{a}	12.2^{b}	12.2^{b}	0.5	0.05	0.03	0.99
Stomach complex,[7] g	951.1	926.9	17.6	1,062.0^{a}	986.9^{b}	844.9^{c}	862.1^{c}	26.9	0.29	<0.01	0.56
g/kg of MBW	20.5	20.2	0.5	20.4^{b}	22.0^{a}	18.0^{c}	21.0^{ab}	0.7	0.51	0.01	0.64
Rumen, g	599.5	587.8	13.7	681.2^{a}	631.6^{b}	530.3^{c}	531.5^{c}	20.8	0.51	<0.01	0.80
g/kg of MBW	12.9	12.8	0.3	13.1^{a}	14.0^{a}	11.3^{b}	13.0^{a}	0.5	0.71	0.01	0.76
Reticulum, g	106.6	106.2	3.8	112.1	112.0	97.4	104.0	5.7	0.93	0.11	0.86
g/kg of MBW	2.31	2.32	0.08	2.15^{b}	2.49^{a}	2.07^{b}	2.54^{a}	0.13	0.90	0.01	0.90
Omasum, g	84.1	82.8	5.2	95.8^{a}	87.1^{ab}	74.8^{c}	76.0^{bc}	6.8	0.79	0.01	0.88
g/kg of MBW	1.97	1.92	0.08	1.92	2.10	1.73	2.01	0.13	0.65	0.14	0.88
Abomasum, g	154.3	145.0	4.9	168.7^{a}	149.2^{b}	136.3^{b}	144.5^{b}	7.5	0.15	0.01	0.59
g/kg of MBW	3.34	3.15	0.11	3.23^{b}	3.33^{ab}	2.90^{c}	3.52^{a}	0.16	0.17	0.01	0.68
Omental fat,[8] g	1,790.5	1,777.7	83.2	2,075.1^{a}	1,696.4^{b}	1,786.0^{b}	1,578.8^{b}	127.0	0.91	0.01	0.73
g/kg of MBW	38.5	38.4	1.8	39.7	37.7	38.1	38.4	2.7	0.97	0.93	0.72
Perirenal fat, g	731.3	861.2	52.1	941.7^{a}	738.1^{b}	784.5^{b}	720.6^{b}	79.5	0.06	0.06	0.69
g/kg of MBW	15.8	18.5	1.0	18.0	16.4	16.8	17.4	1.6	0.04	0.85	0.57
Kidneys, g	108.7	106.7	2.5	118.8^{a}	110.1^{b}	102.8^{bc}	99.2^{c}	3.8	0.54	0.01	0.57
g/kg of MBW	2.35	2.32	0.07	2.28	2.45	2.20	2.42	0.10	0.73	0.13	0.66
Adrenals, g	3.40	3.68	0.60	3.29	3.46	3.17	4.23	0.92	0.72	0.72	0.78
g/kg of MBW	0.07	0.08	0.01	0.06	0.08	0.07	0.10	0.02	0.72	0.32	0.76
Mammary, g	677.6	662.7	58.4	731.9^{a}	755.7^{a}	596.3^{b}	596.6^{b}	76.0	0.78	0.06	0.81
g/kg of MBW	14.5	14.4	1.3	14.2	16.6	12.7	14.4	1.6	0.95	0.19	0.62

[a–d]Means within a row without a common superscript differ ($P \leq 0.10$).

[1]Adequate Se (ASe, 3.05 μg/kg of BW) and high Se (HSe, 70.4 μg/kg of BW) treatments were applied from breeding until slaughter (~d 132 of gestation).

[2]Nutritional treatments were control (C; 100% of maintenance energy requirements) or restricted (R; 60% of maintenance energy requirements). Treatment combinations were: CC (control from d 50 to 130), RC (restricted from d 50 to 90, and control d 90 to 130), CR (control from d 50 to 90, restricted from d 90 to 130), and RR (restricted from d 50 to 130).

[3]Probability values for effects of Se, nutrition, and their interaction.

[4]MBW = maternal BW = final BW − (digesta + gravid uterus).

[5]g/kg of MBW = organ mass (g)/MBW (kg).

[6]Full viscera = stomach complex + small intestine + spleen + pancreas + liver + gall bladder + large intestine, including digesta.

[7]Stomach complex = reticulum + rumen + omasum + abomasum, excluding digesta.

[8]Omental fat is the combined mass of omental and mesenteric fat.

Table 5. Least squares means for maternal intestinal organ mass as influenced by dietary Se and plane of nutrition during mid and late gestation in pregnant adolescent ewes

	Se[1]			Nutrition[2]					*P*-value[3]		
Item	ASe	HSe	SEM	CC	RC	CR	RR	SEM	Se	Nutrition	Se × nutrition
Small intestine,[4] g	456.8	445.0	10.8	484.0^{a}	495.8^{a}	402.6^{b}	421.1^{b}	16.5	0.40	<0.01	0.33
g/kg of MBW[5,6]	9.90	9.71	0.28	9.3^{b}	11.1^{a}	8.6^{b}	10.3^{a}	0.4	0.60	0.01	0.32
Duodenum, g	48.0	46.6	4.2	53.8	51.2	39.3	45.0	6.4	0.80	0.22	0.24
g/kg of MBW	1.04	1.01	0.09	1.03	1.14	0.84	1.09	0.14	0.78	0.34	0.31
Jejunum, g	293.6	289.2	11.3	309.8^{a}	310.8^{a}	271.2^{b}	273.9^{b}	17.3	0.77	0.09	0.99
g/kg of MBW	6.37	6.30	0.26	5.92^{b}	6.94^{a}	5.78^{b}	6.70^{a}	0.40	0.85	0.05	0.94
Mucosa,[7] %	0.64	0.64	0.02	0.66	0.64	0.62	0.65	0.03	0.88	0.64	0.70
Total jejunal mucosa,[8] g	189.1	184.8	9.0	201.9	199.6	167.8	178.5	13.8	0.71	0.11	0.94
g/kg of MBW	4.09	4.03	0.20	3.87^{ab}	4.44^{a}	3.57^{b}	4.36^{a}	0.31	0.83	0.08	0.91
Ileum, g	102.0	93.6	9.1	112.0^{a}	120.0^{a}	79.9^{b}	79.4^{b}	11.8	0.31	0.01	0.15
g/kg of MBW	2.23	2.05	0.20	2.14^{b}	2.70^{a}	1.71^{c}	2.01^{bc}	0.27	0.34	0.01	0.12
Large intestine, g	318.7	311.7	10.3	358.0^{a}	322.6^{b}	276.9^{c}	303.4^{bc}	15.7	0.60	0.01	0.87
g/kg of MBW	6.88	6.78	0.22	6.85^{a}	7.18^{a}	5.89^{b}	7.40^{a}	0.34	0.72	0.01	0.97

$^{a-c}$Means within a row without a common superscript differ ($P \leq 0.10$).

[1]Adequate Se (ASe, 3.05 μg/kg of BW) and high Se (HSe, 70.4 μg/kg of BW) treatments were applied from breeding until slaughter (~d 132 of gestation).

[2]Nutritional treatments were control (C; 100% of maintenance energy requirements) or restricted (R; 60% of maintenance energy requirements). Treatment combinations were: CC (control from d 50 to 130), RC (restricted from d 50 to 90, and control d 90 to 130), CR (control from d 50 to 90, restricted from d 90 to 130), and RR (restricted from d 50 to 130).

[3]Probability values for effects of Se, nutrition, and their interaction.

[4]Small intestine = duodenum + jejunum + ileum, excluding digesta and mesenteric fat.

[5]MBW = Maternal BW = final BW − (digesta + gravid uterus).

[6]g/kg of MBW = organ mass (g)/MBW (kg).

[7]Jejunal mucosa as a percentage of the total mucosal mass.

[8]Total jejunal mucosa = jejunal mass × % jejunal mucosa.

Table 6. Least squares means for DNA, RNA, and protein concentration and total content in jejunum and jejunal mucosa as influenced by dietary Se and plane of nutrition during mid and late gestation in pregnant adolescent ewes

	Se[1]			Nutrition[2]					*P*-value[3]		
Item	ASe	HSe	SEM	CC	RC	CR	RR	SEM	Se	Nutrition	Se × nutrition
Jejunum											
DNA, mg/g	5.50	4.21	0.54	4.90^{b}	3.83^{b}	6.42^{a}	4.27^{b}	0.81	0.07	0.07	0.70
DNA, g	1.63	1.18	0.16	1.50	1.17	1.79	1.16	0.25	0.03	0.12	0.74
RNA, mg/g	6.75	6.24	0.69	5.68	7.01	7.36	5.93	1.05	0.57	0.46	0.55
RNA, g	1.97	1.84	0.23	1.81	2.18	1.96	1.66	0.35	0.65	0.68	0.48
RNA:DNA	1.63	1.66	0.24	1.43	2.01	1.55	1.60	0.37	0.91	0.65	0.17
Protein, mg/g	91.3	78.0	7.2	78.4	87.4	96.7	75.9	10.9	0.16	0.35	0.75
Protein, g	27.0	22.5	2.2	24.2	28.0	25.7	21.1	3.4	0.13	0.41	0.80
Protein:DNA	22.7	20.9	3.0	18.2	29.2	20.9	18.9	4.5	0.63	0.24	0.80
Jejunal mucosa											
DNA, mg/g	6.45	6.50	0.57	7.07^{ab}	5.49^{bc}	7.88^{a}	5.46^{c}	0.87	0.96	0.06	0.45
DNA, g	1.23	1.20	0.12	1.47^{a}	1.08^{ab}	1.35^{a}	0.97^{b}	0.19	0.87	0.08	0.42
RNA, mg/g	8.36	8.24	0.75	7.32^{bc}	9.06^{ab}	10.20^{a}	6.62^{c}	1.14	0.90	0.04	0.25
RNA, g	1.54	1.55	0.16	1.52	1.79	1.70	1.16	0.24	0.93	0.13	0.49
RNA:DNA	1.87	1.49	0.38	1.12	2.04	2.23	1.32	0.58	0.44	0.28	0.16
Protein, mg/g	95.1	90.1	7.0	90.0	98.0	98.6	84.0	10.6	0.59	0.60	0.16
Protein, g	18.5	16.7	1.7	18.8	20.1	16.3	15.2	2.5	0.41	0.35	0.38
Protein:DNA	17.1	15.2	1.6	13.3	20.2	14.9	16.3	2.4	0.36	0.15	0.17

$^{a-c}$Means within a row without a common superscript differ ($P \leq 0.10$).

[1]Adequate Se (ASe, 3.05 μg/kg of BW) and high Se (HSe, 70.4 μg/kg of BW) treatments were applied from breeding until slaughter (~d 132 of gestation).

[2]Nutritional treatments were control (C; 100% of maintenance energy requirements) or restricted (R; 60% of maintenance energy requirements). Treatment combinations were: CC (control from d 50 to 130), RC (restricted from d 50 to 90, and control d 90 to 130), CR (control from d 50 to 90, restricted from d 90 to 130), and RR (restricted from d 50 to 130).

[3]Probability values for effects of Se, nutrition, and their interaction.

Table 7. Least squares means for jejunal tissue cellular proliferation and jejunal vascularity estimates as influenced by dietary Se and plane of nutrition during mid and late gestation in pregnant adolescent ewes

	Se[1]			Nutrition[2]					*P*-value[3]		
Item	ASe	HSe	SEM	CC	RC	CR	RR	SEM	Se	Nutrition	Se × nutrition
Proliferating nuclei, %	10.07	9.39	0.79	8.44	11.59	9.97	8.90	1.21	0.51	0.19	0.81
Jejunum											
Total cells, × 10^9	247.4	178.4	24.7	227.8	177.9	270.4	175.6	37.1	0.03	0.12	0.74
Total cells proliferating, × 10^9	24.9	17.2	3.0	19.2	21.2	27.1	16.6	4.6	0.05	0.25	0.99
Jejunal mucosa											
Total cells, × 10^9	186.3	182.2	18.8	222.0[a]	163.0[ab]	204.9[a]	147.2[b]	28.6	0.87	0.08	0.42
Total cells proliferating, × 10^9	18.4	17.2	2.4	17.7	18.4	21.3	13.7	3.7	0.69	0.35	0.77
Capillary area density,[4] %	6.61	6.81	0.29	6.7	7.0	6.6	6.6	0.4	0.60	0.91	0.43
Capillary No. density,[5] μm^2	0.76	0.73	0.03	0.78	0.74	0.71	0.75	0.04	0.46	0.44	0.55
Capillary surface density[6]	0.05	0.05	0.01	0.05	0.05	0.05	0.05	0.01	0.93	0.42	0.53
Area/capillary,[7] μm^2	95.0	100.3	6.2	91.8	101.2	101.5	96.1	9.4	0.50	0.78	0.54
Total jejunal vascularity,[8] mL	19.4	19.5	1.0	20.2	21.6	17.9	18.2	1.5	0.99	0.17	0.58
Mucosal vascularity,[9] mL	12.0	12.4	0.5	12.3	12.7	11.8	11.9	0.8	0.61	0.87	0.49

[a,b]Means within a row without a common superscript differ ($P \leq 0.10$).

[1]Adequate Se (ASe, 3.05 μg/kg of BW) and high Se (HSe, 70.4 μg/kg of BW) treatments were applied from breeding until slaughter (~d 132 of gestation).

[2]Nutritional treatments were control (C; 100% of maintenance energy requirements) or restricted (R; 60% of maintenance energy requirements). Treatment combinations were: CC (control from d 50 to 130), RC (restricted from d 50 to 90, and control d 90 to 130), CR (control from d 50 to 90, restricted from d 90 to 130), and RR (restricted from d 50 to 130).

[3]Probability values for effects of Se, nutrition, and their interaction.

[4]Capillary area density = (capillary area/tissue area evaluated) × 100.

[5]Capillary number density = (capillary number/tissue area evaluated) × 1,000.

[6]Capillary surface density = capillary circumference/tissue area evaluated.

[7]Area/capillary = capillary area/capillary number per tissue area evaluated.

[8]Total jejunal vascularity = capillary area density (%) × jejunal mass (g).

[9]Total mucosal vascularity = capillary area density (%) × mucosal mass (g).

jejunum and jejunal mucosa were not altered by dietary Se or plane of nutrition.

Maternal Jejunal Cell Proliferation

Least squares means for jejunal cell proliferation variables are detailed in Table 7. At necropsy, the percentage of proliferating nuclei in jejunum was not affected ($P \geq 0.19$) by treatments. As a result of decreased jejunal DNA concentration, HSe ewes had fewer total jejunal cells ($P = 0.03$) and proliferating jejunal cells ($P = 0.05$) compared with ASe ewes. Ewes restricted throughout mid and late gestation (RR) had fewer ($P = 0.08$) total jejunal mucosal cells than CC and CR ewes, although the number of proliferating jejunal mucosal cells did not differ ($P = 0.35$) among plane of nutrition treatments.

Maternal Jejunal Vascularity

Least squares means for maternal jejunal vascularity estimates are presented in Table 7. Vascularity measures such as capillary area density ($P \geq 0.43$), capillary number density ($P \geq 0.44$), capillary surface density ($P \geq 0.42$), and area per capillary ($P \geq 0.50$) were unaffected by dietary Se status or by plane of nutrition during gestation. Despite marked differences in jejunal mass, total vascularity in jejunum ($P \geq 0.17$) and jejunal mucosa ($P \geq 0.61$) was not affected by plane of nutrition treatments.

DISCUSSION

The focus of this experiment was to characterize the key maternal adaptations in response to high dietary Se and nutrient restriction during mid and late gestation and to consider these maternal adaptations in the context of their relationship with fetal growth. It is well known that nutrient restriction or excess during defined periods of gestation can have profound impacts on growth and development of the placenta and fetus (Redmer et al., 2004). In addition, nutritional perturbations that restrict intrauterine growth can have long-term consequences on the health and productivity of offspring during postnatal life (Wu et al., 2006).

Dietary Se concentration of the control diet was adequate according to requirements for gestating ewe lambs (NRC, 1985). Previous studies have documented that supranutritional dietary Se can be fed to sheep without causing signs of Se toxicity (Reed et al., 2007; Juniper et al., 2008). Plasma Se concentrations predictive of chronic selenosis are 2.0 to 3.0 μg/mL (Underwood and Suttle, 2001), which are well above plasma Se concentrations for the HSe ewes (0.62 μg/mL) in this study.

There were dietary Se supplementation by plane of nutrition interactions, indicating that ewe growth responses (ADG and G:F) to Se varied depending on the plane of nutrition. During late gestation (d 90 to 130), HSe increased ADG in CC ewes, whereas HSe decreased ADG and G:F in RR ewes. Previous research has shown

that growth rates of pregnant adolescent ewes (Reed et al., 2007; Neville et al., 2008), growing lambs (Taylor, 2005; Juniper et al., 2008), and growing steers (Lawler et al., 2004) were unaffected by supranutritional levels of dietary Se compared with groups fed adequate amounts of dietary Se.

Although Se is required for cell proliferation (Zeng, 2002), supranutritional dietary Se can potently inhibit cell proliferation by inhibiting DNA synthesis and cell cycle progression as well as by activating apoptosis (Salbe et al., 1990; Yeh et al., 2006; Zeng and Combs, 2008). Although Se supplementation did not affect the proportion of nuclei undergoing proliferation in the jejunum at necropsy, the HSe treatment clearly decreased jejunal DNA concentration. These results suggest that cell proliferation may have been inhibited or that the rate of apoptosis was increased at some point during the supplementation period, thereby decreasing total jejunal cellularity at necropsy.

Reduced jejunal cellularity may have contributed to differences in nutrient absorption, nutrient utilization, or both. Neville et al. (2008) reported that although Se decreased jejunal cellularity, the growth rates of pregnant ewes were not affected by Se supplementation. In the present study, the HSe treatment depressed growth rate in RR ewes only, although jejunal cellularity was decreased by Se with no interaction with plane of nutrition. In addition, the differences in growth performance were most apparent from d 90 to 130. These results suggest that the reduction in jejunal cellularity due to Se supplementation combined with nutrient restriction contributed to impaired nutrient absorption or utilization in RR ewes, but not in ewes that were nutrient-restricted for a shorter period of time (RC and CR).

Nutrient restriction of mid (RC) or late gestation (CR) ewes had similar effects on ADG, which resulted in equivalent final BW at necropsy. However, from d 50 to 130, the RC ewes exhibited greater ADG and G:F than did the CR ewes. This response was clearly driven by the increased G:F of RC ewes during d 90 to 130, during which time the RC ewes were most likely undergoing compensatory growth in response to previous nutrient restriction. These results agree with previous reports in sheep (Kabbali et al., 1992; Freetly et al., 1995) and beef cattle (Sainz et al., 1995) that have demonstrated that ADG and G:F are enhanced during realimentation after a period of undernutrition.

It has been well documented that nutrient restriction markedly decreases the mass of several organs (Wester et al., 1995; Scheaffer et al., 2004a; Reed et al., 2007). Visceral organs such as liver, stomach complex, and small intestine are particularly sensitive to nutrient restriction (Ferrell et al., 1986; Burrin et al., 1990; Reed et al., 2007). Reduced visceral organ mass is a key adaptation to nutrient restriction that contributes to decreased total oxygen consumption by the liver and portal-drained viscera and, ultimately, to decreased maintenance energy requirements (Burrin et al., 1990; Freetly et al., 1995). The CR treatment decreased both total and relative weights of liver, stomach complex, small intestine, and large intestine, which indicates that the mass of these visceral organs was depleted at a disproportionate rate relative to overall BW loss. These data indicate that restriction later in pregnancy can have dramatic affects on visceral organ mass. Due to the marked differences in noncarcass composition between RC and CR ewes at necropsy, it is likely that maternal maintenance requirements per unit of BW were less for CR ewes than for RC ewes. This assertion is supported by work of Ferrell et al. (1986), who reported that lambs fed a low-to-high plane of nutrition had slightly less empty BW, but substantially greater liver, stomach, and intestine weights than did lambs fed a high-to-low plane of nutrition.

Small intestinal cellularity was sensitive to the timing of nutrient restriction as well. The CR ewes had greater jejunal and jejunal mucosal DNA concentration than did RC and RR ewes. Previously, continuous nutrient restriction throughout the last two-thirds of pregnancy did not alter jejunal or jejunal mucosal DNA concentrations (Scheaffer et al., 2004b; Reed et al., 2007). Therefore, it appears that in response to nutrient restriction during late gestation alone, jejunal cellularity of pregnant ewes is maintained or increased despite the marked reduction in jejunal mass. However, the consequences of such physiological adaptations are unclear, particularly in terms of lactation and postnatal offspring performance.

Small intestinal mass as well as jejunal vascularity normally increase throughout pregnancy in sheep (Scheaffer et al., 2004a,b), and nutrient restriction has been shown to potently attenuate jejunal mass and vascularity during pregnancy (Scheaffer et al., 2004a; Reed et al., 2007). Although nutrient restriction consistently decreases jejunal mass and fetal weight in the pregnant ewe (Scheaffer et al., 2004a; Reed et al., 2007), jejunal vascularity is less (Reed et al., 2007) or remains unchanged (Scheaffer et al., 2004b). Therefore, it appears that the reduction in total jejunal mass is more related to reduced fetal growth than is jejunal vascularity.

Regarding the effects of Se supplementation on maternal organ growth, the HSe treatment increased absolute and relative mass of liver and perirenal fat and decreased absolute and relative heart mass without interaction with plane of nutrition. Previous studies that have examined similar concentrations of dietary Se reported no effects on liver mass in growing beef cattle (Soto-Navarro et al., 2004), growing wethers (Taylor, 2005), or gestating sheep (Reed et al., 2007). However, others have shown that Se supplementation increased relative liver mass in gestating ewes (Neville et al., 2008) and growing pigs (Goehring et al., 1984). The increase in maternal perirenal fat in HSe ewes differs from previous research that reported no change due to Se supplementation of pregnant sheep (Reed et al., 2007; Neville et al., 2008). In terms of maternal heart mass, Reed et al. (2007) reported that increased Se intake decreased heart fat mass but had no effect on

total heart mass (g and g/kg of MBW). Whether HSe decreases heart fat mass remains to be verified because it was not measured in the present study.

Selenium supplementation appeared to affect organ growth in a tissue-specific manner (liver, perirenal fat, and heart), which may be related to the degree of Se accumulation in specific tissues. Animal studies have demonstrated that the pattern of Se accumulation differs significantly among various tissues. Taylor (2005) reported that Se concentration in heart and muscle increased linearly, whereas Se concentration of liver responded in a quadratic fashion in wethers fed a high-Se diet for 56 d; however, organ weights were not affected by Se supplementation (Taylor, 2005). In the current study, Se supplementation commenced at breeding and continued until d 132 of gestation; therefore, the longer duration of supplementation may have influenced the degree and pattern of Se accumulation as well as the differences in organ weight. In addition, the metabolic and physiologic adaptations associated with pregnancy represent another significant difference between the current and previous study (Taylor, 2005). The mechanisms underlying the effects of Se on specific organs may be related to effects on cell proliferation. As discussed earlier, in vitro experiments utilizing healthy cell lines have revealed that low concentrations of Se are required to stimulate cell cycle progression and cell proliferation (Zeng, 2002), whereas increased concentrations of Se have been shown to inhibit cell proliferation and stimulate apoptosis (Yeh et al., 2006; Zeng and Combs, 2008). However, Neville et al. (2008) reported that the Se-induced increase in liver weight was associated with greater liver protein concentration but not with changes in liver DNA concentration. These results implicate a possible role of Se in regulation of protein synthesis, as described by Stapleton (2000). Further investigation is required to determine whether tissue Se concentration was related to the tissue-specific responses in total organ weight.

The primary differences between the present and previous studies (Reed et al., 2007; Neville et al., 2008) in pregnant sheep are diet form and means of Se delivery, which may have contributed to different responses. Neville et al. (2008) fed a completely pelleted diet, but Se was supplemented as a selenate-containing aqueous solution or as high-Se wheat. Reed et al. (2007) supplemented a high-Se pellet containing Se-enriched yeast, but the basal diet consisted of chopped alfalfa hay supplemented with a high-Se pellet. In the current study, dietary Se was supplemented in the form of Se-enriched yeast as a portion of a complete pelleted diet. Bioavailability and retention of Se by ruminants is influenced by diet composition as well as chemical form of Se (Koenig et al., 1997; Spears, 2003). For example, Koenig et al. (1997) found that absorption and retention of Se was greater in sheep receiving a concentrate-based diet than in sheep receiving a forage-based diet, although Se retention in the present and previous studies (Reed et al., 2007; Neville et al., 2008) has not been reported. In addition, specific biological effects associated with supranutritional Se intake have been attributed to Se concentration as well as specific Se metabolites (Zeng and Combs, 2008); therefore, comprehensive investigation of the different Se-containing proteins and metabolites may explain different outcomes in the present study compared with previous investigations (Reed et al., 2007; Neville et al., 2008).

In summary, Se supplementation promoted ADG by CC ewes during late gestation (d 90 to 130), but depressed ADG and G:F in RR ewes during late gestation. Possible mechanisms that contributed to the growth depression in nutrient-restricted ewes may be Se-induced reductions in jejunal cellularity, although further research is warranted. Additionally, ewes subjected to nutrient restriction during mid (RC) or late gestation (CR) had markedly different noncarcass composition despite similar BW, which suggests that maintenance energy requirements may be different due to timing of nutrient restriction. The results presented here further emphasize the critical importance of providing appropriate nutrition to pregnant ewes during mid and late gestation.

LITERATURE CITED

AOAC. 1990. Official Methods of Analysis. Vol. I. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Baserga, R. 1985. The biology of cell reproduction. Harvard Univ. Press, Cambridge, MA.

Borowicz, P. P., D. R. Arnold, M. L. Johnson, A. T. Grazul-Bilska, D. A. Redmer, and L. P. Reynolds. 2007. Placental growth throughout the last two thirds of pregnancy in sheep: Vascular development and angiogenic factor expression. Biol. Reprod. 76:259–267.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.

Burrin, D. G., C. L. Ferrell, R. A. Britton, and M. Bauer. 1990. Level of nutrition and visceral organ size and metabolic activity. Br. J. Nutr. 64:439–448.

FDA. 2004. Title 21, Food and Drugs: Food additives permitted in feed and drinking water of animals. http://edocket.access.gpo.gov/cfr_2004/aprqtr/pdf/21cfr573.920.pdf Accessed Dec. 16, 2008.

Ferrell, C. L., L. J. Koong, and J. A. Neinaber. 1986. Effect of previous nutrition on body composition and maintenance energy costs of growing lambs. Br. J. Nutr. 56:595–605.

Finley, J., L. Matthys, T. Shuler, and E. Korynta. 1996. Selenium content of foods purchased in North Dakota. Nutr. Res. 16:723–728.

Freetly, H. C., C. L. Ferrell, T. G. Jenkins, and A. L. Goetsch. 1995. Visceral oxygen consumption during chronic feed restriction and realimentation in sheep. J. Anim. Sci. 73:843–852.

Goehring, T. B., I. S. Palmer, O. E. Olson, G. W. Libal, and R. C. Wahlstrom. 1984. Effects of seleniferous grains and inorganic selenium on tissue and blood composition and growth performance of rats and swine. J. Anim. Sci. 59:725–732.

Jablonka, A., P. M. Fricke, K. C. Kraft, D. A. Redmer, and L. P. Reynolds. 1991. Bromodeoxyurine (BrdU) immunohistochemistry to evaluate labeling index of tissues in vivo. P. Dziuk and M. Wheeler, ed. Handbook of Methods for Study of Reproductive Physiology of Domestic Animals. Sect VII D. Univ. Illinois, Urbana.

Johnson, M. L., D. A. Redmer, and L. P. Reynolds. 1997. Uterine growth, cell proliferation and c-fos proto-oncogene expression

throughout the estrous cycle in ewes. Biol. Reprod. 56:393–401.

Juniper, D. T., R. H. Phipps, D. I. Givens, A. K. Jones, C. Green, and G. Bertin. 2008. Tolerance of ruminant animals to high dose in-feed administration of a selenium enriched yeast. J. Anim. Sci. 86:197–204.

Kabbali, A., W. L. Johnson, D. W. Johnson, R. D. Goodrich, and C. E. Allen. 1992. Effects of compensatory growth on some body component weights and on carcass and noncarcass composition of growing lambs. J. Anim. Sci. 70:2852–2858.

Koenig, K. M., L. M. Rode, R. D. H. Cohen, and W. T. Buckley. 1997. Effects of diet and chemical form of selenium on selenium metabolism in sheep. J. Anim. Sci. 75:817–827.

Lawler, T. L., J. B. Taylor, J. W. Finley, and J. S. Caton. 2004. Effect of supranutritional and organically bound selenium on performance, carcass characteristics, and selenium distribution in finishing beef steers. J. Anim. Sci. 82:1488–1493.

Lu, J., and C. Jiang. 2001. Antiangiogenic activity of selenium in cancer chemoprevention: Metabolite-specific effects. Nutr. Cancer 40:64–73.

Luna, L. G. 1968. Manual of Histologic Staining Methods of the Armed Force Institute of Pathology. 3rd ed. McGraw Hill, New York, NY.

Neville, T. L., M. A. Ward, J. J. Reed, S. A. Soto-Navarro, S. L. Julius, P. P. Borowicz, J. B. Taylor, D. A. Redmer, L. P. Reynolds, and J. S. Caton. 2008. Effects of level and source of dietary selenium on maternal and fetal body weight, visceral organ mass, cellularity estimates, and jejunal vascularity in pregnant ewe lambs. J. Anim. Sci. 86:890–901.

NRC. 1985. Nutrient Requirements of Sheep. 6th ed. Natl. Acad. Press, Washington, DC.

Redmer, D. A., J. M. Wallace, and L. P. Reynolds. 2004. Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. Domest. Anim. Endocrinol. 27:199–217.

Reed, J. J., M. A. Ward, K. A. Vonnahme, T. L. Neville, S. L. Julius, P. P. Borowicz, J. B. Taylor, D. A. Redmer, A. T. Grazul-Bilska, L. P. Reynolds, and J. S. Caton. 2007. Effects of selenium supply and dietary restriction on maternal and fetal body weight, visceral organ mass and cellularity estimates, and jejunal vascularity in pregnant ewe lambs. J. Anim. Sci. 85:2721–2733.

Reynolds, L. P., D. S. Millaway, J. D. Kirsch, J. E. Infeld, and D. A. Redmer. 1990. Growth and in-vitro metabolism of placental tissues of cows from day 100 to day 250 of gestation. J. Reprod. Fertil. 89:213–222.

Sainz, R. D., F. De la Torre, and J. W. Oltjen. 1995. Compensatory growth and carcass quality in growth-restricted and refed beef steers. J. Anim. Sci. 73:2971–2979.

Salbe, A. D., D. Albanes, M. Winick, P. R. Taylor, D. W. Nixon, and O. A. Levander. 1990. The effect of elevated selenium intake on colonic cellular growth in rats. Nutr. Cancer 13:81–87.

Scheaffer, A. N., J. S. Caton, D. A. Redmer, D. R. Arnold, and L. P. Reynolds. 2004b. Effect of dietary restriction, pregnancy, and fetal type on intestinal cellularity and vascularity in Columbia and Romanov ewes. J. Anim. Sci. 82:3024–3033.

Scheaffer, A. N., J. S. Caton, D. A. Redmer, and L. P. Reynolds. 2004a. The effect of dietary restriction, pregnancy, and fetal type in different ewe types on fetal weight, maternal body weight, and visceral organ mass in ewes. J. Anim. Sci. 82:1826–1838.

Soto-Navarro, S. A., T. L. Lawler, J. B. Taylor, L. P. Reynolds, J. J. Reed, J. W. Finley, and J. S. Caton. 2004. Effect of high-selenium wheat on visceral organ mass, and intestinal cellularity and vascularity in finishing beef steers. J. Anim. Sci. 82:1788–1793.

Spears, J. W. 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133:1506S–1509S.

Stapleton, S. R. 2000. Selenium: An insulin-mimetic. Cell. Mol. Life Sci. 57:1874–1879.

Swanson, K. C., D. A. Redmer, L. P. Reynolds, and J. S. Caton. 1999. Ruminally undegraded intake protein in sheep fed low-quality forage: Effect on weight, growth, cell proliferation, and morphology of visceral organs. J. Anim. Sci. 77:198–205.

Taylor, J. B. 2005. Time-dependent influence of supranutritional organically bound selenium on selenium accumulation in growing wether lambs. J. Anim. Sci. 83:1186–1193.

Underwood, E. J., and N. F. Suttle. 2001. The Mineral Nutrition of Livestock. CABI Publ., New York, NY.

Wester, T. J., R. A. Britton, T. J. Klopfenstein, G. A. Ham, D. T. Hickok, and C. R. Krehbiel. 1995. Differential effects of plane of protein or energy nutrition on visceral organs and hormones in lambs. J. Anim. Sci. 73:1674–1688.

Wu, G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84:2316–2337.

Yeh, J. Y., B. R. Ou, Y. C. Liang, J. Burchfiel, J. Butler, N. Forsberg, and P. Whanger. 2006. Mechanism for proliferation inhibition by various selenium compounds and selenium-enriched broccoli extract in rat glial cells. Biometals 19:611–621.

Zeng, H. 2002. Selenite and selenomethionine promote HL-60 cell cycle progression. J. Nutr. 132:674–679.

Zeng, H., and G. F. Combs Jr. 2008. Selenium as an anticancer nutrient: Roles in cell proliferation and tumor cell invasion. J. Nutr. Biochem. 19:1–7.